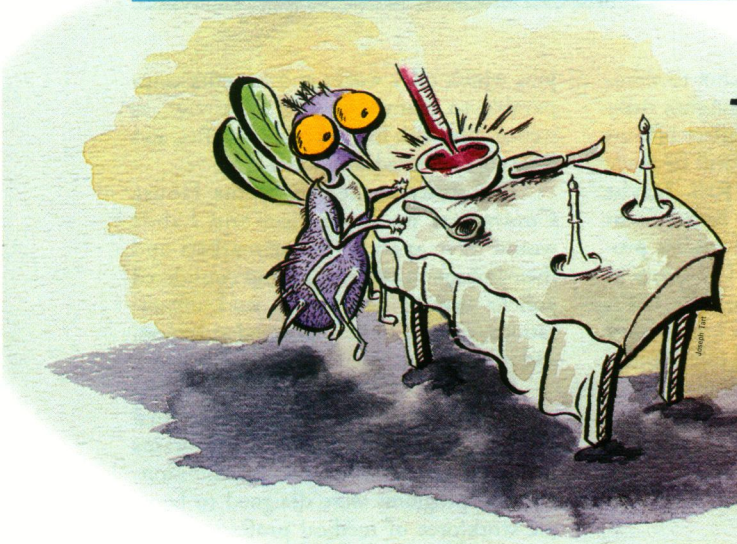


Waiter! There's a Dye in My Soup

First they turn red, then they die. The cause of death is neither envy, rage, nor jealousy. Rather it is a common drug and cosmetic dye, known by its FDA name, D&C Red 28, 27 (water and oil soluble, respectively). The victim of this substance is the fruit fly, long the bane of farmers throughout the world and almost equally the bane of many citizens in areas where the traditional response to infestations of the flies is large scale spraying of malathion, a controversial pesticide.

A Costly Protocol

The sight of fruit flies sends shivers down the spines of farmers who stand to lose millions of dollars whenever they appear. As a group, fruit flies are true to their name in that they primarily attack tree fruit, including plums, peaches, citrus fruits, apples, pears, and cherries. The Mediterranean fruit fly has also been implicated as a pest in grapes, tomatoes, eggplant, and bell peppers, among others.

Under international agreement, anytime fruit flies, including both the Mexican and Mediterranean flies, are found in an area the produce from that region is quarantined from export to uninfested areas. To get rid of this blight, a costly protocol must be followed. First, malathion, a member of the organophosphate family of pesticides, is sprayed. This must frequently be done in populated residential areas. After the spraying, flies sterilized by radiation are released to further control the population.

Although commonly used against fruit flies, malathion presents several basic problems. For one thing, it is toxic to a wide range of living things, although experts are quick to point out that it is not likely to be harmful to humans at the levels used against fruit flies. Nonetheless, in places such as California, where Mediterranean fruit flies are occasionally spotted in residential areas that must then be sprayed, malathion has less than a positive image.

A phototoxic formulation known as SureDye, which is currently undergoing USDA testing, is being investigated as a replacement for, or supplement to, malathion. Because malathion works on contact, it can nonselectively kill desirable insects along with its target pests. "If we have to do a malathion spray against fruit flies, we have to be concerned about disturbing the biological controls for a whole bunch of other pests," said Robert Mangan, supervisory research entomologist at ARS's Weslaco facility, who along with his colleague, research entomologist Daniel Moreno, is a prime mover of the dye testing project.

"Parasites, especially those that control things like California red scale, and woolly white fly...are normally held to very low levels by natural enemies," Moreno explained. "If we repeatedly spray malathion on our orchards, quite frequently it is followed by an outbreak of these other organisms."

SureDye works by ingestion and is composed of the red dye and D&C Yellow 7, 8. The yellow dye is added to the mixture to concentrate light inside a fly's transparent belly and thereby increase the transfer of light energy to the red dye. Chemically, the red dye is phloxine B and the yellow is uranine. They are similar in structure and members of the xanthene chemical group. SureDye is the product of PhotoDye International, a three-year-old company based in Linthicum, Maryland.

Scientists do not know exactly how this seemingly benign dye, used in lipsticks and edible drugs such as antacids, kills flies, but they do know that it must be ingested by a fly and that the satiated fly's gut must then be exposed to some level of light for it to have its toxic effect. Tests indicate that phototoxic dyes can be used selectively against fruit flies, since other organisms lack the transparent guts necessary for light to reach the dye.

Tests of this product and the development of baits to attract flies to it are being conducted at the USDA's Agricultural Research Service (ARS) units at Weslaco, Texas, and Hilo, Hawaii. Most of the tests to date have been in laboratory or small field settings, but the U.S. EPA recently granted experimental use permits to allow for large-scale field tests of the dye on commercial citrus and other crops in Texas, Hawaii, and California.

As part of their phototoxic insecticide research, ARS scientists have identified at least 20 dyes in addition to the SureDye mixture that can kill insects. They haven't looked at the expense or the difficulty involved in registering any of them, but the dyes include several that are approved for human contact of some kind.

Getting Nervous

All organophosphate insecticides, of which malathion is the most popular, kill by effecting the nervous system. They inhibit the function of acetylcholinesterase, an enzyme responsible for cutting off nerve transmissions. When transmissions repeat themselves the body can't cope and wears down.

In the case of humans, if one is "exposed to a very high level of one of these compounds, the ultimate cause of death is respiratory failure," said David Bergsten, a toxicologist, with the USDA Animal & Plant Health Inspection Service in Riverdale, Maryland.

At the level malathion is currently used against fruit flies there is no danger to humans, says Bergsten. Yet, he notes, while "malathion is much more toxic to insects than it is to humans, there still is some inherent toxicity.

"On occasion, as with all compounds, there are certain individuals who may have allergies. . . who may be hypersensitive to various hydrocarbon chemicals, and these individuals may respond at lower levels than one would normally expect," he said. In fact, there have been allegations of human reac-

tion to malathion sprayed against fruit flies, but, says Bergsten, "there have been no proven responses."

Nonetheless, "from a human health standpoint, SureDye is a considerably better compound to be dealing with when you're applying insecticide in areas where humans are likely to be exposed, simply because it is so much less toxic," Bergsten said.

From Death to Birth

SureDye had its genesis during the early 1970s when its developer, James Heitz, was relatively new to the faculty of Mississippi State University, where he is now a professor of biochemistry. While teaching a course on insect biochemistry, a student showed him a *Readers Digest* article titled *Dyed Flies Die*, that resulted from work done on phototoxic dyes by several students at West Virginia University.

"I explained to the student what the biochemical mechanism was for the photodynamic action," said Heitz. "It goes back to the idea that a lot of biochemists knew. If you mixed rose bengal or methylene blue with an enzyme solution and shined light on it you could inhibit the enzyme."

This event spurred Heitz to contemplate a research program on developing pesticides based on this new mechanism. He began by looking at the halogenated xanthenes rose bengal, phloxine B, erythrosin, and easin, and fluoreoscein (which is not halogenated, but is used as a synergist). He found that most of these compounds were approved by the FDA as either food, drug, or cosmetic additives. "So I asked myself, 'What safer pesticide could you develop than a compound that has been consumed by humans for 40 years with no visible side effects,'" said Heitz.

His next step was to round up about 50,000 fire ants from Mississippi's ant-rich soil. He placed them in an aquarium and fed them sugar water laced with rose bengal. "We let them feed overnight in the dark, brought them out into fluorescent light, and lo and behold, they all died," said Heitz. And with their death a new pesticide was born.

Heitz pushed his project full bore until the mid-1980s, but still its time had not yet come. At the time, "malathion looked like the magic bullet that would replace DDT," said Mangan. It has the advantage of breaking down relatively quickly, so it doesn't accumulate in the environment as does DDT.

The phototoxic work lay fallow until Heitz was contacted several years ago by Fred Putsche, Jr., a former Drug Enforcement Administration agent who had used dyes to kill marijuana crops. Research has shown opium and coca are also susceptible. Heitz and Putsche formed PhotoDye to pursue their work. When they contacted Mangan and his ARS colleagues shortly thereafter about a joint research project, a lot remained to be done.

It's Too Salty

Getting the flies to eat the dye was a major hurdle, which was compounded by the different and finicky tastes of a stunning array of species. In all, there are three genera of Tephritidae fruit flies. Two of them—the genus *Anastrepha*, found primarily in North and South America and the genus *Bactrocera*, from Asia—each include an enormous number of species. The genus *Anastrepha* alone accounts for at least 180 species, about 15 of which are economically important.

The Mediterranean fruit fly, which originated in Africa, makes up a genus almost unto itself. "It doesn't have many close relatives of great concern. . . . It seems to do all its damage by itself," said Mangan, who noted that it is of more concern to the United States than any other fruit fly species.

The primary concern in Texas is the Mexican fruit fly, or *Anastrepha ludens*. California, because of its Pacific Coast exposure, is victimized by 20 to 30 fruit fly species. Although neither Texas nor California has a permanent tropical fruit fly population, Hawaii's economy is severely impacted by quarantines due to persistent infestations of several types of fruit flies.

The first attempt at getting fruit flies to eat SureDye fell completely flat. It was at Weslaco, where Mangan and Moreno mixed the dye with bait commonly used with malathion against the Mediterranean fruit fly in California, and tried to get Mexican fruit flies to eat it. But with malathion, the flies don't have to eat bait. All they need do is be attracted by it, and then touch the poison. It turned out that the flies, which have taste sensors on their feet, found the bait entirely too salty. Even in captivity, when given nothing else to eat, some flies would rather die than eat the bait, while those that ate the bait died of a salt overdose.

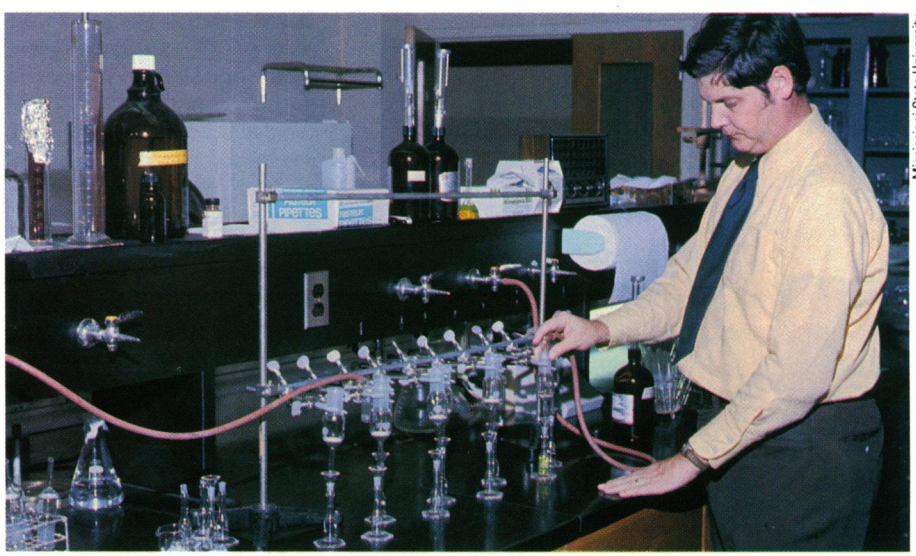
Like Bad Beer

Undaunted, the scientists continued their work with the conviction that "we could kill 95–99% of adults in a habitat with a spray program using this dye," said Mangan. After a year of experimentation, the ARS/PhotoDye team joined forces with the California Department of Food and Agriculture, which was searching for a malathion replacement and had funding to pursue that goal.

At about the same time, Corn Products of Summit, Illinois was looking for a market for a by-product of the fermentation process it uses to produce corn starch and syrup. The company asked the ARS to test the by-product, called Mazoferm, as a conventional fruit fly trap bait.

Instead, Mangan and his colleagues decided to see if Mexican fruit flies might like this "brownish yellow liquid, that smells like really bad beer," with an eye toward using it as a SureDye bait. And the flies went for the brew. "In fact, they consumed enormous amounts of it," said Mangan. Further research revealed that the flies liked it even more if high fructose sugar was added to the Mazoferm. The current experimental bait consists of about 70% Mazoferm, 10% high fructose sugar, 1% SureDye, 1% of a surfactant to speed incorporation of the dye into the insect, and the rest water.

Meanwhile in Hawaii, entomologist Nicanor Liquido and his colleagues at the ARS's Tropical Fruit and Vegetable Research Laboratory in Hilo, have been developing a different bait for the Mediterranean fruit fly, which has proved to be a fussy eater.



Mississippi State University

Dye developer. James Heitz works on SureDye in his laboratory at Mississippi State University.

At first they tried mixing SureDye with various hydrolyzed protein taste enhancers, such as those used in frozen foods and dried soup preparations. The flies liked the smell and showed up for dinner, but they did not eat. The Hawaiian researchers then tried a hydrolyzed protein prepared from the fermentation of yeast, rather than corn from which Mazoferm is brewed, and, says Liquido, "They love the smell, and they love to eat it."

Out to Dinner

One of the beauties of using phototoxic dyes is that they take advantage of fruit fly lifestyles. The flies generally enjoy a morning bask in the sun, for example. So that after a nice colorful repast, this traditional diversion spells certain death. This also helps prevent the deaths of desirable insects that may not like the bait, or are not sun worshipers.

The dyes also avail themselves of fruit fly mating habits. As part of their courtship behavior the male fly takes the female "out to dinner," in a manner of speaking, prior to copulation. Specifically, the males, who tend to forage more than the females, like to dance around on a leaf while fluttering their wings and spitting. The female coyly happens by to consume what the male has spit and is soon the object of his amorous advances. "We saw this as a way of more efficiently feeding it to the females. That was another reason we got so excited," said Mangan.

Tests on flies in the laboratory, in outdoor cages, and in limited field tests, have given the USDA scientists good reason to be excited. At the Weslaco facility, four days of feeding with the SureDye mixture killed 95% of the Mexican fruit fly population. And limited field testing on 10 acres heavily infested with Mediterranean fruit flies in Hawaii, reduced the population by 50% even though these 10 acres were surrounded by thousands of untreated acres.

It will now be up to the more extensive field tests recently approved by the EPA to determine if, in their natural habitat where they can eat whatever they please, the flies will go for the phototoxic bait.

Scott R. Bauer/USDA-ARS

Toxic takeout. Entomologist Nicanor Liquido places a dye and bait mixture on cotton wicks on which flies can be observed while ingesting the insecticidal meal.

Roll Over

Despite considerable research into the question of how phototoxic dyes kill insects, the answer is still frustratingly evasive. While the dyes must be ingested to be toxic, "We don't think the target organ is the gut," said Mangan. "We suspect it's some other organ, maybe the nervous system. . . because we found that the dye acts much faster if we add surfactants that make the gut more permeable, and get it out into the hemolymph."

Another indication that the nervous system may be involved is the way in which the flies die. They don't spend their last moments buzzing around in circles then making a beeline for the floor, as when sprayed with a household insecticide. Rather, they walk around a bit, roll over on their sides, and stop moving.

Of the chemical mechanism by which the ingested dye operates, Heitz says, "The dye itself doesn't kill anything. Instead, it absorbs light energy and gives it off to oxygen molecules in the cells. It takes the ground state oxygen, and raises it to an excited state called singlet oxygen, which is a very good oxidizer. . . . It keeps generating this singlet oxygen as long as light is hitting the insect."

Having a liver and bile duct is what saves humans and other animals from the effects of phototoxic dyes. "The liver filters these dyes out of the blood stream," Heitz explains. The dyes then go through the bile duct, into the intestine, and are excreted from the body. Additionally, the internal organs of animals function in total darkness, hence they are protected from any light-catalyzed reaction.

Speculation as to when phototoxic dyes may enter the commercial arsenal used against fruit flies is difficult, although ARS's Liquido says that "If all our pilot tests are successful, I think there will be both scientific and political force to expedite registration." There is already considerable political support for the dyes. Representative Ken Calvert (R-California,) who is a member of the House agriculture committee, lent his support to obtaining EPA's issuance of an experimental use permit for SureDye which will allow further testing. According to Dave Ramey, a member of Calvert's staff, the California delegate has a longstanding commitment to finding alternatives to malathion and "[SureDye] seemed like one of the better ideas out there."

But even if it is eventually found that there are some as of yet unforeseen problems with phototoxic dyes, all of the research will not have been for naught. As Mangan explains it, "There are lots of good insecticidal products that have to be ingested by insects, but nobody has advanced them because we never took the time to figure out how to make the bugs eat them. We know how to do that now." For fruit flies it seems, now when the dinner bell rings, they won't have to ask for whom the bell tolls.

Victor Chase

Suggested Reading

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